Bladder dysfunction during Spinal Cord Injury: Functional changes reflected in the organ morphology

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1. Abstract

Spinal cord injury (SCI) affects many organs and their function, including the urinary bladder. The consequence of SCI is neurogenic lower urinary tract dysfunction (NLUTD), in which communication between cerebral circuits and the urethral sphincter is disrupted, leading to spontaneous contractions of the bladder. If left untreated, it can lead to devastating consequences such as hypertrophy, fibrosis and ultimately renal failure. Tumor necrosis factor-alpha (TNF-a), which is mainly produced by macrophages, has been shown to be involved in fibrotic and hypertrophic responses. The aim of this thesis is to detect macrophages and to investigate morphological changes in SCI mouse bladders. During the SCI experiment, bladder function was evaluated in the animals from the test (SCI) group and the control (sham-operated) group. Masson's Trichrome staining was carried out on the frozen sections to observe collagen formation in the bladder tissue and immunohistochemical (IHC) staining was done to examine different cell types, including immune cells, especially macrophages. The results show that the collagen content in SCI mouse bladders of the test group were significantly increased compared to the control group.

2. Introduction

The urinary bladder is a muscular hollow organ lined with urothelium and is part of the lower urinary tract (LUT). The main functions of the bladder are the storage and controlled release of urine. [1]
Many neuronal diseases, including the spinal cord injury (SCI), can cause neurogenic lower urinary tract dysfunction (NLUTD). SCI disrupts micturition by interrupting communication between the cerebral circuits that coordinate the activity of the bladder and the urethra. The bladder contracts spontaneously and involuntarily which causes dangerously high bladder pressure peaks resulting in bladder wall fibrosis and renal failure if left untreated. [2] TNF-α, which is primarily produced by activated monocytes/macrophages, is also relevant in fibrotic diseases. It has been shown that exposure of the bladder to TNF-α causes fibrotic and hypertrophic changes, which can lead to ultimately loss of contractility. [3]

3. Aims/ Leading Questions

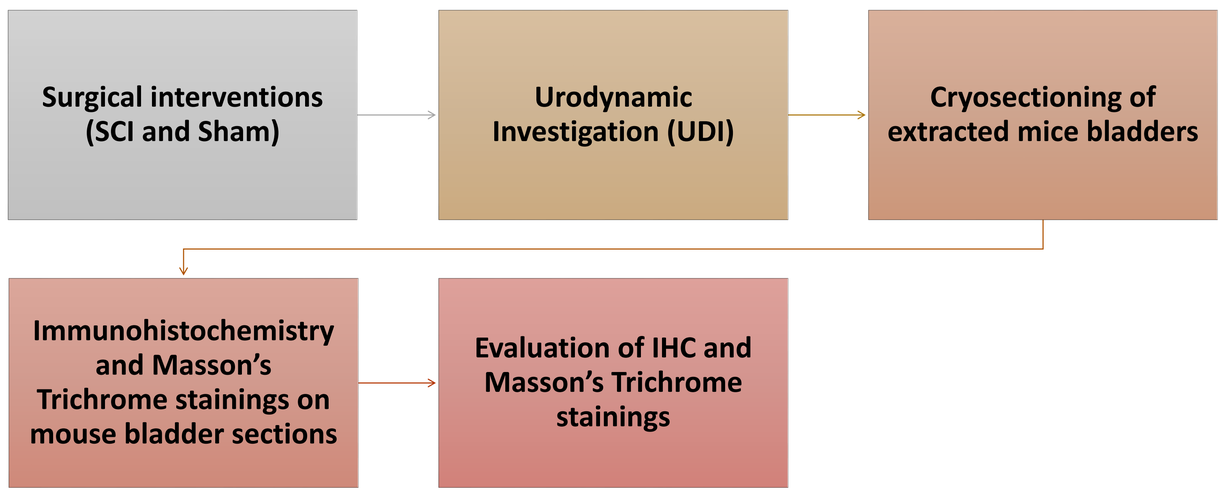
The aim of this project is to find a link between bladder contractility, determined by urodynamic examination and bladder fibrosis, and investigate the presence of tissue macrophages.

1. How much fibrosis occurs in the bladders of the mice with experimentally induced SCI?
2. Can we detect an increase of macrophages in the bladders of SCI mice?
3. Which morphological characteristics do SCI mouse bladders and bladders of control mice undergoing urethral sphincter electrode implantation have? Where are the differences?

References
[1] AMBOSS. (2019). Harnblase. Retrieved from https://www.amboss.com/de/wissen/Harnblase
[2] Birkhäuser et al., (2020). TASCI-transcutaneous tibial nerve stimulation in patients with acute spinal cord injury to prevent neurogenic detrusor overactivity: Protocol for a nationwide, randomised, sham-controlled, double-blind clinical trial. BMJ Open. 10(8). 1-10.
[3] Koeck, I., Hashemi Gheinani, A., Baumgartner, U., Vassella, E., Bruggmann, R., Burkhard, F. & Monastyrskaya, K. (2018). Tumor Necrosis Factor-α Initiates miRNA-mRNA Signaling Cascades in Obstruction-Induced Bladder Dysfunction. American Journal of Pathology. 188(8). 1847-1864.

Figures
Fig. 1 Gashi, B. (2021). Ratio of collagen to total bladder tissue of the Sham and SCI group. Bern: own figure
Fig. 2 Gashi, B. (2021). Infiltration level of the Sham and SCI group stained with F4/80 antibody. Bern: own figure

4. Material and Methods



5. Results

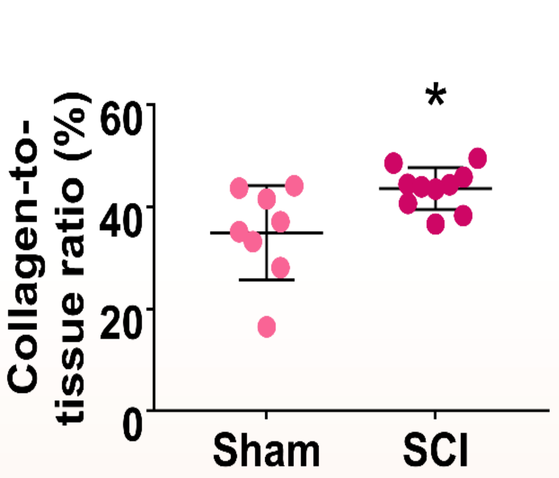


Fig. 1 Ratio of collagen to total bladder tissue of the Sham and SCI group. (Gashi, 2021)

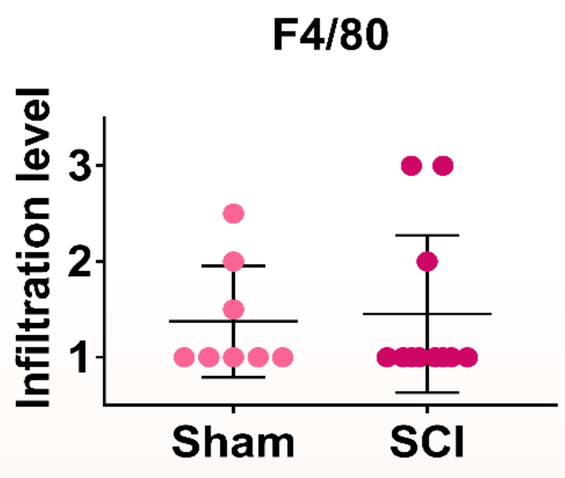


Fig. 2 Infiltration level of the Sham and SCI group stained with F4/80 antibody. (Gashi, 2021)

Fig. 1 shows the ratio of collagen to the total stained bladder section of SCI and Sham. A significant difference (p-value = 0.017) was detected between the two groups, indicating higher levels of collagen in the tissues of the test group compared to the control group.

Fig. 2 shows the infiltration level of the samples from the Sham and SCI groups, stained with the F4/80 antibody. Two samples in the SCI group have an increased probability of infiltration. In the control group (Sham), individual mouse bladders have a moderate to increased chance of infiltration. However, most Sham-samples were rated at level 1.

Level 1: low to zero probability of being F4/80 positive cells.
Level 2: medium probability of being F4/80 positive cells.
Level 3: high probability of being F4/80 positive cells.

6. Discussion

Histological examination in mice with SCI
The statistical analysis of the trichrome stainings did show a significant difference between the SCI mouse bladders and the control mouse bladders. The collagen-to-tissue ratio presents an increase of collagenous tissue in the test group.

Histological examination of the IHC stainings from SCI
The macrophage antibody (F4/80) did not work clearly on the SCI mouse bladder sections. The fact that the actin antibodies also reacted to the same cells, which should not happen to macrophages, means that it cannot be confirmed that staining is specific. There were some exceptions where the cells were stained only with the F4/80 antibody, but this was a very small amount in relation to the number of samples.